Faculty of Resource Science and Technology

Effect of Incubation Period on Fermentation of *Petai Belalang* Seeds for Coffee-Like Beverage Production

Claudia ak Richard Beginda

(50449)

Bachelor of Science with Honours
(Resources Biotechnology Programme)
2018

Effect of Incubation Period on Fermentation of *Petai Belalang* seeds for Coffee Production

Claudia ak Richard Beginda (50449)

This project is submitted in partial fulfillment of the requirement for degree of Bachelor of Science with Honours
(Resource Biotechnology)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2018

ACKNOWLEDGEMENT

First and foremost, praise given to God for giving me the willpower, patience and good health throughout the completion of this final year project. I would like to express my most sincere appreciation and gratitude to my greatly supportive supervisor, Dr Dayang Salwani bt Adenan for her constant guidance and consultation along the completion of my project. My special thanks to all postgraduates in the laboratory especially Mr Hemnaarth Tenakaran for his patience and guidance as well through it all. My gratitude also goes out to all final year project students under Dr Dayang's supervision which are my laboratory mates and my laboratory partner under this topic, Nurul Nadiah bt Ahmad Daud for all their patience, support and contributions throughout this project. I would also like to give credits towards Dr Ho Wei Seng for providing me with the genetically modified lowered-mimosine version of the Petai Belalang seeds. My special appreciation dedicated to my examiner for giving me a valuable guidelines and opinions for writing this report. Last but not least, I am deeply grateful to my family members especially my parents for their encouragement, concern and financial supports.

UNIVERSITI MALAYSIA SARAWAK

Grade: ____________

Please tick (√)

Final Year Project Report ☐

Masters ☐

PhD ☐

DECLARATION OF ORIGINAL WORK

This declaration is made on the 4th day of JUNE year 2018

Student's Declaration:

I CLAUDIA AK RICHARD BEGINDA, 50449, FACULTY OF RESOURCE SCIENCE AND TECHNOLOGY (PLEASE INDICATE NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled, EFFECT OF INCUBATION PERIOD ON FERMENTATION OF PETAI BELALANG SEEDS FOR COFFEE-LIKE BEVERAGE PRODUCTION, is my original work. I have not copied from any other students' work or from any other sources with the exception where due reference or acknowledgement is made explicitly in the text, nor has any part of the work been written for me by another person.

15 JUNE 2018
Date submitted

CLAUDIA AK RICHARD BEGINDA (50449)
Name of the student (Matric No.)

Supervisor's Declaration:

I, DR DAYANG SALWANI BT ADENAN (SUPERVISOR'S NAME), hereby certify that the work entitled, EFFECT OF ~~FERMENTATION PERIOD ON~~ INCUBATION PERIOD ON FERMENTATION OF PETAI BELALANG SEEDS FOR COFFEE-LIKE BEVERAGE PRODUCTION (TITLE) was prepared by the aforementioned or above mentioned student, and was submitted to the "FACULTY" as a * partial/full fulfillment for the conferment of BACHELOR OF SCIENCE WITH HONOURS (RESOURCE BIOTECHNOLOGY) (PLEASE INDICATE THE DEGREE TITLE), and the aforementioned work, to the best of my knowledge, is the said student's work

Received for examination by: ____________
(Name of the supervisor)

Date: 4/6/18

I declare this Project/Thesis is classified as (Please tick (√)):

☐ CONFIDENTIAL (Contains confidential information under the Official Secret Act 1972)*
☐ RESTRICTED (Contains restricted information as specified by the organisation where research was done)*
☑ OPEN ACCESS

I declare this Project/Thesis is to be submitted to the Centre for Academic Information Services and uploaded into UNIMAS Institutional Repository (UNIMAS IR) (Please tick (√)):

☑ YES
☐ NO

Validation of Project/Thesis

I hereby duly affirmed with free consent and willingness declared that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abide interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic and research purposes only and not for other purposes.
- The Centre for Academic Information Services has the lawful right to digitize the content to be uploaded into Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis if required for use by other parties for academic purposes or by other Higher Learning Institutes.
- No dispute or any claim shall arise from the student himself / herself neither a third party on this Project/Thesis once it becomes the sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student himself/herself without first obtaining approval from UNIMAS.

Student's signature ______ (4/6/18) (Date)

Supervisor's signature: ______ 4/6/18 (Date)

Current Address:
NO 337B, LORONG 6A9, TAMAN MATANG JAYA, JALAN MATANG, 93050, KUCHING, SARAWAK.

Notes: * If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organisation with the date of restriction indicated, and the reasons for the confidentiality and restriction.

[The instrument was prepared by The Centre for Academic Information Services]

DECLARATION

I hereby declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification to this or any other university or institution of higher learning.

(CLAUDIA AK RICHARD BEGINDA)

Resource Biotechnology

Department of Molecular Biology

Faculty of Resource Science and Technology

University Malaysia Sarawak

Effect of Incubation Period on Fermentation of *Petai Belalang* seeds for Coffee Production

Claudia ak Richard Beginda (50449)

Resource Biotechnology
Faculty of Science and Technology
University Malaysia Sarawak

ABSTRACT

This study focused on fermentation of *Leucaena leucocephala* or *Petai Belalang* seeds using distilled water to investigate the effects of different incubation period that gives the optimum concentration of starch and protein and shows the highest sugar level within the seeds. In this experiment, genetically-modified, lowered-mimosine version of the seeds are fermented under different incubation period which is 6 hours, 12 hours, 18 hours and 24 hours. For every incubation period, there are triplicate of samples are fermented together with the same weight which is 5 g of seeds each and 15 mL of distilled water as working volume under the inoculum size of 15% w/v. In total, there are 12 samples fermented under the same inoculum size, which in this study yeast plays the role as the inoculum. The concentration of starch and protein obtained is compared to seeds that settle in the same working volume and weight on similar incubation period but without the addition of yeast in the media as positive control. Sugar level is also determined through DNS method. In this study, the incubation period that gives the highest sugar level is at 18 hours. The selection of sample to run the DNS test is based on the concentration of starch since it is a form of simple sugar. At inoculum size of 15% w/v, starch analysis of sample at the 18^{th} hours give sugar content of 0.2896 g/L. Meanwhile the lowest glucose level is detected at the 6^{th} hours, which is 0.1828 g/L. From the study, it can be concluded that different incubation period affects the analysis of starch and protein as well as the sugar level of the seeds after being fermented by using yeast.

Key words: *Leucaena leucocephala,* incubation period, inoculum size, sugar level, starch content.

ABSTRAK

Kajian ini menfokuskan proses penapaian atas biji Petai Belalang menggunakan air sulingan bagi mengenalpasti kesan jangka masa pengeraman yang akan memberi kepekatan optimum kanji dan protein serta paras gula tertinggi dalam biji tersebut. Dalam eksperimen ini, biji yang telah dimodifikasi dari paras genetiknya dan mempunyai kandungan mimosin yang telah direndahkan melalui proses penapaian dibawah jangka masa pengeraman yang berbeza iaitu pada 6 jam, 12 jam, 18 jam dan 24 jam. Bagi setiap jangka masa pengeraman, terdapat tiga sampel yang mempunyai berat yang sama iaitu 5 g melalui proses penapaian menggunakan jumlah air 15 mL dibawah saiz inokulum 15% w/v. Secara keseluruhannya, terdapat 12 sampel yang ditapai menggunakan saiz inokulum sama, dimana yis merupakan inokulum tersebut dalam kajian ini. Kandungan kepekatan kanji dan protein yang diperoleh menerusi analisa dibandingkan dengan biji yang hanya diletakkan dalam air tanpa melalui proses penapaian kerana tiada sebarang penambahan yis dilakukan namun bertindak sebagai kontrol positif. Kandungan gula juga boleh ditentukan menerusi kaedah DNS. Dalam kajian ini, jangka masa pengeraman yang memberi kandungan gula tertinggi adalah pada biji yang melalui proses penapaian yang berlangsung selama 18 jam. Pemilihan sampel untuk menjalankan ujikaji DNS adalah berdasarkan kandungan kepekatan kanji dalam sampel. Ini adalah kerana kandungan kanji mempengaruhi nilai gula yang terkandung. Dengan menggunakan saiz inokulum 15% w/v, analisa kanji atas biji yang telah melalui jangka masa pengeraman 18 jam memberikan paras gula pada 0.2896 g/L. Dalam pada itu, paras gula terendah dapat dikenalpasti pada sampel yang melalui jangka masa pengeraman 6 jam sahaja, iaitu 0.1828 g/L. Kesimpulannya, kajian ini menunjukkan bahawa jangka masa pengeraman yang berbeza dalam proses penapaian boleh mempengaruhi analisa kanji dan protein serta paras gula dalam biji Petai Belalang.

Kata kunci: Petai Belalang, jangka masa pengeraman, saiz inokulum, paras gula, kepekatan kanji

TABLE OF CONTENTS

List of Abbreviations

%	Percentage
°C	Celsius (temperature)
IUCN	International Union for Conservation of Nature
FFTC	Food and Fertilizer Technology Centre
US	United States
kg	Kilogram
MARDI	Malaysia Agricultural Research and Development Institute
EU	European Union
MKL1	Polyhybrid clone
MKR1	Polyhybrid clone
spp.	Species (plural)
pH	Power of Hydrogen
DPPH	2,2'-diphenyl-1-picrylhydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
UV	UltraViolet
g	Gram
mL	Millilitre
cm	Centimeter
rpm	Revolutions Per Minute
OD	Optical Density
g/L	Gram per Liter
nm	Nanometer
BSA	Bovine Serum Albumin
G250	G = stands for greenish hue
mg/mL	Milligrams per millilitre
ANOVA	Analysis of Variance
M	Molarity

P	Probability
NaOH	Sodium hydroxide

List of Tables

List of Figures

1.0 Introduction

In Malaysia, the coffee industry is still conducted in small scales due to our beans production productivity. This is due to our geographical condition that does not favors towards the growth of Arabica beans, but instead of Liberica beans that has lower product quality values in terms of the cup qualities. The main coffee production location in Malaysia is in Johor, Sabah, and Selangor as reported by FFTC Agricultural Policy platform (2016). Petai Belalang seeds as a potential for coffee substitutes has already been discovered and pioneered in certain countries. Having an alternative does not completely substitute the local coffee industry, but rather, it adds or expand wider range of coffee-like beverage as an export stock. Vegetarian foods are an epitome for the situation as their existence does not changes the status of normal poultry in the market for example. But vegetarian products provide another type of choice to cater with consumer's preference. Globally, coffee is known as one of the cash crop. However, in Malaysia the crops are competing with other vegetation that are being placed prior such as oil palm resulting in the less progressive cultivation activity by the locals. Though cultivation are normally carried out in remote area by villagers. The petai belalang trees that are categorised as invasive plants are abundantly available. While the labor expenses for coffee production are high due to the harvesting process, the petai belalang seeds on the other hand can be easily obtained. In another context, through this study it can be made possible that product values can be added to petai belalang seeds and in turn contributes to the socio-economic development of the people that lives in rural areas.

The problem statement of this study is to find out if induced fermentation using yeast culture on this *L. leucocephala* seeds will be able to improve the sensory attributes such as acidity, bitterness, and aroma of the yielded beverage similar as in common coffee fermentation. This study hypothesized that the fermented product from the seeds of *L. leucocephala* will have more refined sensory attributes after being roasted as compared to the products from non-fermented seeds.

1.1 Research objectives:

I. To investigate the effect of incubation period on fermentation of *Petai Belalang* seeds for production of coffee.

II. To investigate the incubation period that determines the optimum starch to produce coffee-like beverage.

2.0 Literature review

2.1 Coffee Production Over the World

As the third most consumed beverages choices after plain water and tea, coffee also tops as the second most traded commodities worldwide next to petroleum oil. According to the USDA Foreign Agricultural Service (2017), the coffee world markets and trade forecasted that the production of coffee is at 159 million bags (60 kilograms) and has remained constant for several years. In August 2017, trade statistic shows that the exports value of coffee has heaped to 9.87 million (International Coffee Organization, 2017). There was also expectation on the world exports of green coffee beans to remained at 111 million bags. Meanwhile, the global consumption for the beverage are forecasted to be at 158 million bags, worldwide.

The International Coffee Organization has listed ten nations as the world's top coffee producer. Ranked as the tenth, Guatemala's coffee production is amounted 204,000 thousand (60 kilograms) in 2015. The coffee beans production is reportedly to be high when the temperature is in the range of 16-32°C provided that the altitude is at 500 and 5000 metres above sea level (Top Coffee Producing Countries, 2015). Another top coffee producing country that are ranked in the fourth is Indonesia. The country is well known for producing the world's most expensive coffee that are collected from palm civets. "Kopi Luwak" or the "Cat Poop Coffee" are sold up to 80 US Dollar that equals to 339 Ringgit Malaysia when converted. However, only 800 kg of these Kopi Luwak beans are produced annually. On the other hand, Vietnam are very known for their Vietnam coffee ranked the second highest coffee producing nation. USDA Coffee Foreign Agricultural Service (2017) reported that the coffee production value in Vietnam are affected by the drought that took place in 2016 hence the value is still recovering from the incident. The statistics by the USDA Coffee Foreign Agricultural Service however shows that the production value of coffee beans has been climbing up due to sufficed rainfall amount throughout the first three month of the year 2017 and are forecasted to amounted on 28.6 million. Vietnam tops the production of Robusta beans with 27.5 million bags in 2017. As for Brazil, ranked at number 1 for the top coffee producer in the world, the plantation area took up approximately 27,000 square kilo of the country itself (Top Coffee Producing Countries, 2015). Brazil also produces the topnotch Arabica beans and the output are forecasted to amounted at 40.5 million bags (USDA Coffee Foreign Agricultural Service, 2017). Brazil, Vietnam and Colombia also topped the chart for the total exports over the world. Meanwhile, for the domestic consumption record, European Union has topped the chart with the amount of 44.8 million for 60 kg bags of coffee beans. The European Union (EU) is also the highest coffee importer with the record of 40%, next to the United States.

2.2 Coffee Industry in Malaysia

The coffee production in Malaysia ranked at the 60th globally as reported by Nor and Mohd., (2016). Therefore, the country does not contribute any significant percentage to the world's coffee production. Nor and Mohd., also reported that this is due to Malaysia's coffee beans growth are leaned more towards the Liberica beans, although there are also Arabica beans cultivation but in a much smaller scale and less favorable. The Liberica beans comprised most of the coffee cultivation area since it was the first coffee crop introduced to Malaysia back in the days (Nor & Mohd, 2016). The lower quality of Liberica beans production does not really contribute any significant impact in the country's export rate, hence Malaysia are deemed as more of the importer instead. Another reason for this was that, Malaysia is a country that focuses more on other commodities such as palm oil and petroleum gas especially, creating a more intense competition for coffee against these cash crops.

Likewise, in many other Asean countries, the demand on Robusta coffee are high in Malaysia for the use in instant coffee products. According to the International Coffee Organization (2014), the coffee consumption of 2012 in East and Southeast Asia shows that total consumption of the beverage in Malaysia only totalled up to 393 thousand bags and 0.8 kilograms for per capita consumption. This implies a negative domestic consumption, as reported by the International Coffee Organization.

The largest instant coffee plant in Malaysia are the Super Coffeemix plant that are located in Johor. Coffee as commodity or cash crop in Malaysia gives a major impact in the welfare of the rural communities (FFTC Agricultural Policy Platform, 2016). In the country, Malaysian Agricultural Research and Development Institute (MARDI) are the ones that held accountable for the research and development framework for the local coffee cultivation. As reported by the FFTC Agricultural Policy Platform, MARDI has launched clone for Liberica, MKL1 and a poly-hybrid Robusta Coffee, MKR1 both in the year of 1992.

2.3 Coffee Fermentation

The fermentation process in coffee plays a significant role in the removal of mucilage layer from coffee beans. The mucilage layer comprises of 84.2% water, 8.9% protein, 4.1% sugar, 0.9% pectic substances and 0.7% ash as reported by Belitz, Grosch & Schieberle (as cited by Lee et al., 2015). According to Avallone et al., (2006), the alcohol insoluble component of the mucilage polysaccharide composition contains 30% pectin, 8% cellulose and 18% neutral non-cellulose polysaccharide that furthermore consists of monosaccharides

(Arabinose, xylose and galactose) as well as simple sugars.The metabolism process of these components that took place during fermentation in wet-processing specifically is theorised to be responsible for the detachment of the mucilagenous layer from the coffee cherries. Coffee fermentation are naturally happening due to presence of microorganisms. Gram negative bacteria such as Klebsiella spp., and Erwinia spp., are among the microflora involved in industry fermentation process in coffee along with several pectinolytic yeast species. These microorganisms grow on glucose. Fermentation aided with these microflora contributes in the acidity of coffee. This acidic environment induces the available starch to produce insoluble sediments. At the end of fermentation, only pectinolytic yeast would survive as they are resistance towards the very low pH. However, there are no increase in the number of pectolytic microflora throughout fermentation as the bacteria will be using the pectic breakdown's product after metabolizing the polysaccharide. The end-point for fermentation can be observed when the initial inoculum is equal as the biomass of the product.

2.3.1 Effect of Incubation Period on Coffee Fermentation

Incubation period also plays a major role in fermentation as it correlates with the number of starch and protein at the end of fermentation. Fermentation period are also affected by the degradation of mucilage. Velmourougane (2012) reported that Arabica coffee beans take shorter time which are 13 hours for complete mucilage removal as compared to Robusta beans that took up to 98 hours due to its thicker mucilage layer. Initial stage of coffee beans fermentation always kicks off with abundance of yeast and bacteria that are directly proportional with the high amount of sugar from mucilage. Presence of yeast will inhibit mold formation (Velmourougane, 2012). Velmourougane also stated that the fermentation duration directly affects the cup qualities as flavor component can be lowered by high amount of fungi. In Arabica coffees, it took up to 20 hours to obtain a good coffee characteristic. A cup quality can be measured based on the Hedonic scale.

2.4 Formation of Coffee Aroma

Roasting of coffee beans are one of the most highlighted avenue as it is the biggest influences for aroma of coffee. Aroma plays a major role that sets as standards in consumer perception for a coffee product. Formation of coffee aroma took place during roasting of the beans which is due to the Maillard reaction at temperature more than 200 °C (Lee et al., 2015). Caramelized products are also formed at this stage due to the thermal degradation of starch and simple sugars as also reported by Lee et al. Maillard reaction produces coffee aroma compounds such as pyrazines, thiols and furanones. Different odorant compound gives off different sensory description. For example, the phenolic compound vanillin gives off vanilla-like sensory and 2,3-butanedione gives off buttery and caramel-like flavour. At the same time, chrogenic acid and non-volatile phenolic compounds are hydrolised during the reaction producing derivatives that will form potent-volatile phenolic compound. In 2008, Wong, Abdul Aziz and Mohamed also proved that Maillard reaction between certain amino acids and glucose will produce sensory profiles based on the characteristic of amino acids involved.

2.6 *Petai Belalang*

Petai Belalang is known as *Leucaena Leucocephala* or the scientific name for the plant are *Leucaena leucocephala* (Lam.) de Wit ssp. *ixtahuacana* C.E. Hughes (Hughes, 1998). The physical profile for the plant are small and shrub trees that are highly branched. They also have bipinnate leaves and produces flowers that are white in colour, making it an angiosperm. The mode of reproduction for the plant are self-fertilize that allows flowering and seeding takes places all year long. The seeds of *Petai Belalang* can remain viable for atleast 20 years long in the soil and germination occur over a lengthened period (Global Invasive Species Database, 2017). *L. leucocephala* or *Petai Belalang* is a fast-growing and a nitrogen fixing trees as it is from the family Leguminosae. The tree was also reported by Agroforestry Species Profile (2017) to be able to resist forest fire. In addition to that, *L. leucocephala* are listed as the least concern species in the IUCN red list and described as weed or invasive plants. Listed as category II weed, *Petai Belalang* trees are known to pose a risk to the native vegetation as it can aggresively colonizes the area.

The common use for the tree is used as a shade plant for crops such as coffee and cocoa. The leaves are normally given to fed livestocks. The seed of the *petai belalang* can be produced up to 15,000 to 20,000 per kilograms and is a potential substitute for coffee. The *Petai Belalang* trees are native to many tropical as well as subtropical countries which gives them the ability to tolerate with wide range of temperature.

2.6.1 *Petai Belalang* Seeds as Coffee Substitute

The use of *petai belalang* seeds as coffee substitute has been pioneered in several continents but only the country of Philippine has properly documented the plant for its product values. In the Philippines, *Leucaena leucocephala* are known as "*Ipel-Ipel*" in Tagalog. Despite of being recognized more as weed for its invasive behavioral, the plants are still deemed as worth for cultivating due to the reason that it belonged to the legume family. This means that the plants have the attributes of nitrogen-fixing and any legumes are the major contributor to the nitrogen cycle. Brill (1916), suggested that the seeds of the *ipel* produce the same colour and odour when being roasted and brewed. According to Brill, the infusion is perceived as brown in colour when observed under direct light, also there was no precipitate formed when treated with acid and base. However, the test with litmus paper changes the blue coloured litmus into red when being dipped into the infusion. In the Philippine Journal of Science, Brill also furtherly reported that the density and impurity of the infusion were being compared to coffee at 15.56 °C by treating it with ten times its weight of cold water, boiled and then filtered. When being treated in such manner, the ground seeds are found to be soluble and gives off 24.4% of extracts as well as density 1.00680.

	Ipel.		Coffee.	
	Raw.	Roasted.	Raw.	Roasted.
Moisture	14.80	8.13	10.73	2.38
Fat (ether extract)	8.68	6.95	11.80	13.85
Nitrogen	6.42	5.21	2.32	2.60
Sucrose	trace	trace	7.62	1.31
Nitrogen free material other than fiber	9.78	16.11	20.30	39.38
Crude fiber	22.29	22.18	24.01	18.07
Ash	4.20	4.37	3.02	4.65

Figure 1. Analyses of Ipel and Coffee. Adapted from "Ipel, a coffee substitute Leucaena glauca (LinÃƒâ€šÃ‚-naeus) Bentham" by H. C. Brill., 1916, *Philippine Journal of Science Vol. 11A*(3), Pp 101-104.

	Index of refraction.	Saponification.
Ipel:		
Raw	1.4670	196.2
Roasted	1.4690	190.7
Coffee:		
Raw	1.4695	157.2
Roasted	1.4715	162.6

Figure 2. Oil contents in Ipel and Coffee. Adapted from "Ipel, a coffee substitute Leucaena glauca (LinÃƒâ€šÃ‚-naeus) Bentham" by H. C. Brill., 1916, *Philippine Journal of Science Vol. 11A*(3), Pp 101-104.

2.6.2 Constituents of the raw seeds of *Petai Belalang* (%)

Table 1. *Constituents of the raw seeds of Leucaena leucocephala.*

Fat	8.68
Crude Fiber	22.59
Nitrogen-free material	9.78
Nitrogen	6.42
Water	14.81
Ash	4.2
Crude protein	46
Carbohydrates	45
Calcium	4.4

Note. Adapted from *Ipil-ipil, Leucaena glauca, Santa Elena, Yin he huan : Herbal Therapy / Alternative Medicine in the Philippines* by StuartXChange, 2002. Retrieved from http://www.stuartxchange.org/Ipil-ipil.html

Seeds also contain sucrose and 15% fatty acid with saponification value 108.74 as well as 1.08 acid value as reported by Stuart (2002). The seeds of *petai belalang* gives off high antioxidant activity from its flavanoidal constitutents. They also contain high phenolic content compared to the stems and the leaves of the trees.

3.0 Materials and Methods

3.1 Materials

3.1.1 *Petai Belalang* seeds

Genetically modified with reduced mimosine level *petai belalang* seeds are collected from another laboratory in UNIMAS. The seeds are washed and oven-dried each time before using. 5 g of the seeds are used for each triplicate in all the batches and is placed in 15 mL of distilled water as the working media.

3.1.2 Inoculum

Baker yeast or Mauripan yeast contained *sacchromyces cerevisae* are used in this study. Instead of adding the yeast directly into the media, they are first added into autoclaved Yeast Malt Broth and diluted with distilled water by five times dilution factor for plating on Potato Dextrose Agar. The inoculum size used for this study is 15%. 2.25 mL of the solution is used to made up the 15% inoculum size. Only the pellets obtained from centrifugation is added into the media and is diluted using the working volume itself.

3.1.3 Yeast Malt Broth

Yeast Malt Broth is available in the laboratory and is used to cultivate yeast. The broth contain sodium propionate and diphenyl that can eliminate mould to prevent presence of mixed population.

3.1.4 Potato Dextrose Agar

Potato dextrose agar are available in the laboratory and is used to make agar plates.